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Kamal Patra as an Antidote in *Dhatura* Poisoning in Albino Mice - In Vivo Study

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ABSTRACT

Introduction: *Dhatura (Dhatura metel)* is cerebrotoxic, Deleriant poison which is also classified as *Upavisha* in *Ayurveda*. Various poisons have been described in *Ayurveda* along with their antidotes. Mode of action of these antidotes is not mentioned in texts. In *Ayurvedic* literature According to *Basavrajeeyam* under the heading of *Vish-Prativishani*, *Chincha Rasa* and *Kamal Patra churna* have been described to be possessing antidote action which may act by some way to counter toxicity of *Dhatura*. It is necessary to verify the efficacy of these antidotes on scientific parameters so that it can be useful in emergencies.

Objectives: To establish the action and mechanism of *Kamal Patra Churna* against toxic effects of *Dhatura* on albino mice. Methods: Swiss albino mice were selected as an animal model and antidote potential of kamal patra against datura poweder is evaluated by measuring body temperature, time of convultions and survival rate.

Results: Kamal patra administration before datutal poisoning reduced the toxic effects of datura such as hypothermia, convulsion and increased the survival rates.

Conclusion: From this study, we can conclude that *Kamal Patra* can resist the toxic effects of *Dhatura* up to some extent.

Key Words: *Dhatura, Kamal Patra, Agad*, Toxicity, Antidote

INTRODUCTION

In the ancient era, *Ayurveda* was considered as one of the advanced faculty worldwide. *Agadtantra* is the branch of *Ayurveda* which deals with the toxicity of various snakes, spiders, insects, rats etc. animals and its treatments. The word '*Gada*' means poison and the antidote used is called as 'Agada'.[1] According to modern science, Toxicology is the branch which deals with the study of poison regarding their sources properties, mode of action, symptoms which they produce, lethal dose, fatal period, treatment of their detection estimation & autopsy findings.[2,3] *Dhatura* (*Dhatura metel*) is cerebrotoxic, Deleriant poison which is also classified as *Upavisha* in *Ayurveda*.[4,5]

It is a genus of poisonous herbs shrubs, up to the height of 3–4 ft. This plant has been noted for intoxicating, narcotic properties, they produce temporary insensibility (stupefying effects) in ordinary doses. A bitter taste, dryness of mouth and throat, burning pain in the stomach dysphasia, headache and difficulty in talking are the first symptoms that are complained of. These are followed by giddiness, staggering gait, in the co-ordination of the muscles, the peculiar flushed appearance of the face, dry hot skin, photophobia, dilated pupils, delirium & drowsiness. Sometimes, exfoliations of the skin are seen over most of the body. The pulse becomes irregular and intermittent. In fatal cases drowsiness passes into stupor convulsions, coma, death occurs usually from respiratory failure.

Various poisons have been described in *Ayurveda* [6-8] along with their antidotes. These antidotes are readily available in nature. Mode of action of these antidotes is not mentioned in texts. In *Ayurvedic* literature according to *Basavrajeeyam*

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under the heading of *Vish-Prativishani*, *Chincha Rasa* and *Kamal Patra churna* has been described to be possessing antidote action which may act by some way to counter toxicity of *Dhatura*.[9-11] *Kamal* (*Nilumbo nuciphera*) is a beautiful aquatic plant with a wide range of medicinal usage. It is *Madhura, Tikta Rasatma*k and *Shita Virya* hence reduces *Pitta Dosha*.[12-14] Main chemical constituents of *Kamal* are Nuciferine,10-Nonacosanol, Nelumboside, Neferine, Nuciferin, Nelumbine Quecitring,

It is necessary to verify the efficacy of these antidotes on scientific parameters[15] so that it can be useful in emergencies. Hence present study entitled "*Kamal Patra* as an Antidote in *Dhatura* poisoning in mice was carried out to establish the action and mechanism of *Kamal Patra Churna* against toxic effects of *Dhatura* on albino mice.

MATERIAL AND METHODS

Collection of material

Seeds of *Dhatura metel* were collected in December to January so at that time seeds can be collected from dried fruit.

The collection of leaves of *Kamal* (*Nelumbo nucifera*) for the study was done. Collected leaves were dried in shade. The samples were used in powder form.

Procedure

1. *Dhatura metel*: 1 gm coarse powder of *Dhatura* seeds *choorna* were extracted with 70% alcohol in the warm water bath with occasional shaking for ½ hr. in closed tubes, successively with the batches of 3,2 and 1 ml of alcohol. 10µl of the pooled extract was spotted on TLC plate silica gel F_{254}, Merk and was developed in Toluene: ethyl Acetate: Diethylamine (7:2:1).

Detection of Rf Values of the spot was done by using 5% methanolic sulphuric acid reagent. Rf value of visualized spots was given in table no.7

2) *Nelumbo nucifera*: 1 gm coarse powder of *Kamal Patra Choorna* were extracted with 70% alcohol in the warm water bath with occasional shaking for ½ hr. in closed tubes, successively with the batches of 3,2 and 1 ml of alcohol .10µl of the pooled extract was spotted on TLC plate silica gel F_{254}, Merk and was developed in Toluene: methanol: pyridine (8:1:1). Rf value of visualized spots was given in table no.8 and 9.

Animal Experiment

Material for In Vivo study

Swiss Albino mice of age 90-100 days and weight ranges 20-25 gm were procured. Poisoning was induced by *Dhatura* seed and test drug was kamal patra powder.

Determination of acute toxicity of Dhatura

Acute toxicity test (LD_{50} determination) of the extract of *Dhatura* seeds is performed as per guideline of OECD in Swiss Albino mice.

Determination of protective action of Kamal Patra against toxicity of Dhatura seed

Reduction in mortality and change in behaviour of mice due to a lethal dose of *Dhatura* seed after administration of *Kamal* leaves are determined.

Dose Calculation

Dose Calculation for Albino Mice

The conversion factor from man to mice is 0.0026 so according to this, all the doses were calculated. Dose for mice was obtained from the following formula

Dose of mice = 0.0026 X Dose of Man

Dose Calculation for Dhatura Seeds

Human fatal Dose for *Dhatura* Seeds is considered as 100 – 120 crushed seeds i.e. calculated as 1400 mg. According to the conversion factor, fatal dose in albino mice for *Dhatura* is 3.64 mg

Hence per kg wt. Fatal dose for albino mice = 03.64 x 50

= 182 mg/kg

Dose Calculation for Kamal Patra Powder

Human therapeutic dose of *Kamal Patra* is considered as 3 gm – 6 gm. According to the conversion factor, fatal Dose in albino mice for *Kamal Patra* is 7.8 to 15.6 mg

The dose I: Per kg wt. dose for albino mice = 7.80 x 50

= 390 mg/kg

Dose II: Per kg wt. dose for albino mice = 15.6 x 50

= 780 mg/kg

Procedure

Drug samples were converted into suspension by careful mixing with distilled water. Both drugs were administered orally. After administering the dose all animals were observed for 24 hours for toxic sign and symptoms or mortality up to 7 days. First preliminary drug toxicity study for *Dhatura* was done. In each group weight of each animal was taken and noted, simultaneously dose of *Dhatura* and *Kamal Patra* were calculated accordingly. Samples of Toxic drug and Antidote were given by oral route. After dosing; the animals were observed for 24 hrs and up to 7 days. Comparative observations were tabulated (Table 2).

Group I: Acute toxicity of *Dhatura* Seed *churna* (N=6)

Group II: *Kamal Patra churna* (Dose 1) + *Dhatura* Seed *churna* (N=6)

Group III: *Kamal Patra churna* (Dose 2) + *Dhatura* Seed *churna* (N=6)

Parameters:

i) Change in Temperature
ii) Appearance of Convulsions
iii) Dilatation of Pupils
iv) Survival period(death)

RESULTS AND DISCUSSION

Analytical Study

Laboratory experiments were carried out to obtain values of following parameters for *Dhatura* seeds, *Kamala Patra* the observation and results follow (Table 1-5)

Table 1: Loss on drying and Ash value

1. Loss on drying

Samples	Wt. of samples at room temp.	Wt. of samples after drying at 105°	Percentage loss on drying at 105°
1. *Dhatura* seeds	2.80 gm	2.60 gm	5.86%
Kamal Patra	1.45 gm	1.37 gm	6. 2 %

2. Ash Value

Samples	Total ash	Water-soluble ash	Acid insoluble ash
1. *Dhatura* seeds	2.73%	0.32%	0.23%
2. *Kamal patra*	0.9%	0.13%	0.05%

Table 2: Alcohol and Aqueous extractive values at Dhatur seeds, Kamal Patra

Alcohol extractive values at Dhatur seeds, Kamal Patra

Samples	Alcohol extractive values
1. *Dhatura* seeds	6.39%
2. *Kamal Patra*	10.72%

Showing Aqueous extractive values Dhatura seeds, Kamal Patra

1. *Dhatura* seeds	6.32%
2. *Kamal Patra*	17.68%

Table 3: Showing preliminary phytochemical screening of Dhatura seeds

Sr. No.	Plants constituents	Test/reagent	Dhatura seeds	
			Alcohol extract	Water extract
1.	Steroids	Salkowski reaction Liebermann-Burchard	+ ve - ve	+ ve - ve
2.	Alkaloids	Dragendorff's reagent mayer's reagent	+ ve + ve	+ ve + ve
3.	Tannin	Ferric chloride test 5% lead acetate test	- ve - ve	- ve - ve
4.	Flavonoids	Shinoda test	-ve	- ve
5.	Carbohydrates	Molish's test Barfoets test	+ ve - ve	+ ve - ve
6.	Amino acids	Ninhydrin test	- ve	- ve
7.	Proteins	Biuret test	+ ve	+ ve

Table 4: Phytochemical screening of sample of Kamal Patra (Nelumbo nucifera)

S. No	Plant constituents	Test	Result
1	Test for carbohydrate	A) Molisch test	(+)
2	Test for steroids	A) Salkowski test B) Libermann test	(+)
3	Test for Tannin	A) Ferric chloride test B)Lead acetate test	(-)
4	Test for Alkaloids	A)Dragendorff's test B)Wagner's test C)Hager's test D)Mayer's test	(-)
5	Test for Flavonoids	A)Shinoda test	(+)
6	Test for Proteins	A)Biuret test	(-)
7	Test for Saponin	A)Foam test	(-)
8	Test for Glycosides	A) General test B) Killer killiani test C)Legal test	(-) (-) (-)

Effect of poisoning and kamal patar in animals

Changes in body Temperature

The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes (Table 6A and B). The mean change in body temperature in group 1 was 38.14±0.43. While in Group 2 it reduced

Table 5: HPTLC [Planner chromatography of Dhatura seeds]

Peak	Start Rf	Start Ht	Max Rf	Max Ht	Max %	End Rf	End Ht	Area	Area %	Assigned Substance
1.	0.02	5.7	0.02	39.5	4.71	0.03	21.8	253.2	2.28	
2.	0.03	6.6	0.04	450.0	53.62	0.08	3.3	3784.8	34.04	Thiamine (B_1)
3.	0.08	4.5	0.09	25.4	3.03	0.10	0.1	176.10	1.58	Peptide
4.	0.17	0.2	0.20	21.1	2.52	0.21	13.4	291.7	2.62	
5.	0.21	14.1	0.23	24.3	2.90	0.25	1.80	400.1	3.60	
6.	0.32	7.8	0.36	20.0	2.38	0.37	17.9	603.8	5.43	Carbohydrate
7.	0.39	15.6	0.42	43.8	5.22	0.44	9.3	1005.9	9.05	Carotenoids
8.	0.45	9.6	0.46	15.3	1.83	0.48	3.8	228.8	2.06	
9.	0.49	0.3	0.52	20.3	2.42	0.52	17.5	383.3	3.45	Peptide
10.	0.53	17.8	0.53	20.1	2.39	0.56	4.7	275.0	2.47	
11.	0.56	4.9	0.59	27.0	3.22	0.63	6.3	736.6	6.62	Peptide
12.	0.74	1.7	0.78	27.4	3.27	0.82	0.3	644.9	5.80	Vit. A
13.	0.83	0.9	0.85	10.2	1.21	0.90	0.0	182.2	1.64	
14.	0.96	0.0	0.99	94.7	11.29	1.02	39.4	21.53.7	19.37	Etioporythyrin

to 37.44±0.37 and further in Group 3 it reduced down to 37.08±0.33. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1 with Group 2 and Group 2 with Group 3 suggested statistically Non Significant changes. While Comparison of Group 1 with Group 3 suggested Mean difference of 1.0630 and Q value 4.798 which is statistically significant.

Table 6A: Changes in body temperature (°C) after Acute toxicity of *Dhatura* in Albino mice (n=6)

Group	Mean±SD	SEM	P-value	Inference
Gr. -1	38.14 ±0.43	0.25	0.0378	
Gr. – 2	37.44 ±0.37	0.31		Significant
Gr. –3	37.08±0.33	0.19		

Table 6B: Showing Tukey Kramer Multiple comparison tests within the groups

Comparison	Mean difference	Q value	P-value	Inference
Gr.-1 vs Gr.-2	0.6967	3.144	> 0.05	Not significant
Gr.-1 vs Gr.-3	1.0630	4.798	< 0.05	significant
Gr.-2 vs Gr.-3	0.3667	1.655	> 0.05	Not significant

Time of the appearance of Convulsions

The observations were analyzed statistically by one way ANOVA Test. The P-value suggested Non-significant changes (table 7A and B). The mean change in the time of appearance of Convulsion in group 1 was 153.32±7.33. While in Group 2 it increased to 159±4.91 and further in Group 3 it increased up to 166.43±874. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested Non-significant changes. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1, Group 2 and Group 3 suggested statistically Non-Significant (Graph 1,2).

Table 7A: Time of appearance of Convulsions (Min) after Acute toxicity of Dhatura in Albino mice (n=6)

Group	Mean±SD	SEM	P-value	Inference
Gr. -1	153.32±7.33	4.23		
Gr. – 2	159.32±4.91	2.85	0.1617	Not Significant
Gr. – 3	166.43±8.74	5.04		

Table 7B: Showing Tukey Kramer multiple comparison test within the groups

Comparison	Mean difference	Q value	P-value	Inference
Gr.-1 vs Gr. - 2	-6.000	1.448	> 0.05	Not Significant
Gr.-1 vs Gr. - 3	-13.107	3.163	> 0.05	Not Significant
Gr.-2 vs Gr. - 3	-7.107	1.715	> 0.05	Not Significant

Time of Dilation of Pupil

The mean change in the time of dilatation of Pupils in group 1 was 111.89±6.79. While in Group 2 it increased to 119±6.04 and further in Group 3 it increased up to 133.58±7.57. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1 with Group 2 and Group 2 with Group 3 suggested statistically Non-Significant. Comparison of Group 1 with Group 3 suggested Mean difference of 21.79 and Q value 5.50 which is statistically Significant. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes (Table 8A, B).

Table 8A: Time of Dilation of Pupil (Min) after Acute toxicity of Dhatura in Albino mice (n=6)

Group	Mean±SD	SEM	P-value	Inference
Gr.-1	111.89±6.79	3.92		
Gr.–2	119.75±6.04	3.48	0.0217	Significant
Gr.–3	133.58±7.57	4.37		

Table 8B: Showing Tukey Kramer Multiple comparison tests within the groups

Comparison	Mean difference	Q value	P-value	Inference
Gr.-1 vs Gr.-2	-7.870	1.99	> 0.05	Not Significant
Gr.-1 vs Gr.-3	-21.69	5.50	< 0.05	Significant
Gr.-2 vs Gr.-3	-13.82	3.50	> 0.05	Not Significant

Duration of Survival period

The mean change in the time survival Period (Death) in group 1 was 370.36±24.99. While in Group 2 it increases to 402.35±17.88 and further in Group 3 it increases up to 424.51±22.72. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested Non-significant changes.

Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1, Group 2 and Group 3 suggested statistically Non-Significant. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1 with Group 2 suggested Mean difference of 31.99 and Q value 2.51 which is statistically Non-Significant. Comparison of Group 1 with Group 3 suggested Mean difference of 54.15 and Q value 4.25 which is statistically Non-Significant. Comparison of Group 2 with Group 3 suggested Mean difference of 22.16 and Q value 1.73 which is statistically Non-Significant.

The observations were analyzed statistically by one way ANOVA Test. The P-value suggested Non-significant changes (Table 9A and B).

Table 9A: Duration of Survival period (Min) after Acute toxicity of Dhatura in Albino mice (n=6)

Group	Mean±SD	SEM	P-value	Inference
Gr. – 1	370.36±24.99	14.42		
Gr. – 2	402.35±17.88	10.32	0.0623	Not quite significant
Gr. – 3	424.51±22.72	13.12		

Table 9B: Showing Tukey Kramer multiple comparison test within the groups

Comparison	Mean difference	Q value	P-value	Inference
Gr.-1 vs Gr.- 2	-31.99	2.51	> 0.05	Not Significant
Gr.-1 vs Gr.- 3	-54.15	4.25	> 0.05	Not Significant
Gr.-2 vs Gr.- 3	-22.16	1.73	> 0.05	Not Significant

CONCLUSION

Dhatura is neurotoxic cerebral deliriant poison which is also classified as *Upavisha* in *Ayurveda* with '9 Ds' toxic effects. The active principle is dhaturine containing hyoscine, hyocymine and atropine. It blocks the acetylcholine receptors and thus produces sympathomimetic or parasympatholytic actions (Anticholinergic actions). In vivo study of Antidote properties of *Kamal Patra* shows, hyperthermia caused due to toxicity is significantly reduced, duration of the appearance of convulsions is increased slightly but it is statistically insignificant, duration of dilatation of the pupil is significantly increased, rise in duration if the survival period is statistically insignificant but a slight rise was seen in it.

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Conflicts of Interest

There are no conflicts of interest

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